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TITLE: UV-Induced Epigenetic Field Effect as a Target for Melanoma Therapy and Prevention

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14. ABSTRACT

Cutaneous malignant melanoma is a complex, aggressive, and highly chemoresistant cancer that arises from the pigment producing melanocyte cells located within the skin. It has a high mutational burden associated with exposure to ultraviolet radiation (UVR). UV- induced DNA mutations (C>T) are incontrovertibly linked to the disease; however, the most common mutations found in both nevi and melanoma (i.e. BRAFV600E and NRASQ61L/R) do not bear UV-signature mutations. It is unclear if these are initiators or selected for during disease progression highlighting our lack in knowledge of the critical molecular targets in the initiation of UV-induced melanoma. We propose an alternative hypothesis that UV-driven epigenetic reprograming of a field of melanocytes (or an epigenetic field-effect) proceeds and complements subsequent DNA mutations in the progression of melanoma. We aim to understand the involvement of these proposed epigenetic changes in the underlying molecular mechanisms of UV-induced melanoma. This would be the first evidence epigenetic alterations from UV-induced stress in mammalian cells. These findings could ultimately identify potential new-biomarkers for excessive sun-exposure that could be used to assess an

15. SUBJECT TERMS

Skin-cancer, melanoma, ultraviolet-radiation, epigenetics, methylation, genetics, melanomagenesis, melanocyte, environmental-carcinogens, sunburn

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1. **INTRODUCTION:**

Cutaneous malignant melanoma is a complex, aggressive, and highly chemoresistant cancer that arises from the pigment producing melanocyte cells located within the skin. It has a high mutational burden associated with exposure to ultraviolet radiation (UVR). UV-induced DNA mutations (C>T) are incontrovertibly linked to the disease; however, the most common mutations found in both nevi and melanoma (i.e. $BRAF^{V600E}$ and $NRAS^{Q61L/R}$) do not bear UV-signature mutations. It is unclear if these are initiators or selected for during disease progression highlighting our lack in knowledge of the critical molecular targets in the initiation of UV-induced melanoma. We propose an alternative hypothesis that UV-driven epigenetic reprograming of a field of melanocytes (or an epigenetic field-effect) proceeds and complements subsequent DNA mutations in the progression of melanoma. We aim to understand the involvement of these proposed epigenetic changes in the underlying molecular mechanisms of UV-induced melanoma. This would be the first evidence epigenetic alterations from UV-induced stress in mammalian cells. These findings could ultimately identify potential new-biomarkers for excessive sun-exposure that could be used to assess an individual's risk and help define personalized prevention strategies.

2. **KEYWORDS:**

Skin-cancer, melanoma, ultraviolet-radiation, epigenetics, methylation, genetics, melanomagenesis, melanocyte, environmental-carcinogens, sunburn

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
- Major Task 1: UV irradiation of mice and isolation of melanocytes
    - Subtask 1: Produce iDct-GFP mouse crosses and irradiate pups (1-8 mo) (100%)
    - Subtask 2: FACS-isolate melanocytes from mouse skin 30-days post irradiation (1-8mo) (20%)
    - Subtask 3: Isolation of DNA and perform DREAM analysis (9-14mo) (20%)
    - Milestone: Epigenetic data analysis completed (14mo) (70%)
- Major Task 2: Validation of specific genes

- Subtask 1: Identification of specific genes epigenetically modulated by UV radiation in skin melanocytes and also found to be similarly modulated in melanoma tissues (14-16mo) (0%)
- Subtask 2: Assess the biomarker utility of identified epigenetic gene signatures (16-22mo) (0%)
- Subtask 3: Proof of principle experiments to show that epigenetic drugs can reverse the UV-induced epigenetic expression modulation (20-24mo) (0%)
- Milestone: UV-modulated epigenetic biomarkers identified and validated in human samples (24mo) (0%)

- Major Task 3: Test whether UV irradiated melanocytes are susceptible to transformation by genetically manipulating the melanocytes isolated 30-days post *in vivo* UV irradiation
  - Subtask 1: Isolate melanocytes from UV-irradiated mouse skin using iDct-GFP mice (8-12mo) (100% completed)
  - Subtask 2: Genetic manipulations of UV irradiated vs control melanocytes with expression of oncogenes $NRAS^{G12V}$ and $BRAF^{V600E}$, suppression of tumor suppressor p53 via dominant negative $p53^{R248W}$, and suppression of the RB pathway via active $CDK4^{R24C}$ (12-18mo) (20%)
  - Milestone: Validate the hypothesis that UV irradiated melanocytes are more prone to transformation by specific oncogenes (18-24mo) (30%)
- **What was accomplished under these goals?**

Major Task1:
Major Activities completed: The iDct-GFP mouse crosses have been completed and one litter have been irradiated so far. These crosses include iDct-GFP on the C57B6 background with and without cBrd allele that produces an albino mouse. Melanocytes have begun to be collected from the mouse skin and are being saved for isolation of DNA for subtask 3. DNA for all samples for major task 1 are still being collected, after which DREAM will be performed along with data analysis and comparison with previous mouse and human datasets. This should be completed before the timeline.

Major Task2:
Major activities completed: This task is dependent on the completion of the first task and will begin once it is completed

Major task 3:

Major Activities completed: Melanocytes have been isolated from UV-irradiated and control iDct-GFP mice. Genetic manipulations of these cells has been attempted. We have obtained all plasmid constructs for the proposed genetic manipulations along with successful packaging by a lentivirus. We have not been able to culture the melanocytes after isolation which was an expected problem so we will continue with the proposed alternative approach to the method.

- **What opportunities for training and professional development has the project provided?**
  - Contributor, Sarah Preston (Graduate Student), has received training and professional development while working on this project. This includes training in mouse modeling and techniques in isolation of melanocytes with Dr. Zaidi. She has worked closely with the other contributors of this project, Jaroslav Jelinek and Jozef Madzo, who have trained her in analysis and statistical interpretation of the data-sets obtained including work with R statistical programming and use of publicly accessible databases. This training will continue for the entirety of the project. Skills in technical writing and scientific presentations have developed through creation of materials such as poster presentations, seminar meetings, and grant applications.
- **How were the results disseminated to communities of interest?**
  - We have presented preliminary results through poster presentations at two events: Dawn Mark's Day and at Fels Trainee Day. Additionally, one oral presentation was given to the Fels Institute at Temple University.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - We plan to complete major task one which includes completion of DREAM analysis of our samples. This will allow us to begin working on major task 2. We will identify genes that have been epigenetically modified by UV-radiation and in melanoma tissues along with testing the use of epigenetic changes as possible biomarkers. For major task 3 we will identify the method of culturing primary melanocytes and by the end of reporting period we should have completed most of this task.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  - The findings of this study are likely to develop the understanding of how UV-induced changes in melanocytes that cause melanoma focusing on non-mutational changes. These findings could identify potential new-biomarkers for excessive sun-exposure that could be used to assess an individual's risk and help define personalized prevention strategies.
- **What was the impact on other disciplines?**

  Nothing to report

- **What was the impact on technology transfer?**
  - Nothing to report
- **What was the impact on society beyond science and technology?**
  - Nothing to report

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
  - *Nothing to report*
- **Actual or anticipated problems or delays and actions or plans to resolve them**
    - Culture of primary melanocytes from the skin has been difficult due to excessive cell death; however, we anticipated this and will continue with our alternative approach to culture melanocytes from mice that contain Ink4a/Arf-/- or Pten-/- to see if this leads to better survival of the cells.
- **Changes that had a significant impact on expenditures**

  Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  Nothing to report

- **Significant changes in use or care of human subjects**

Nothing to report

- **Significant changes in use or care of vertebrate animals.**

Nothing to report

- **Significant changes in use of biohazards and/or select agents**

Nothing to report

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**

Presentation of the data:

Dawn Mark's Day, Lewis Katz School of Medicine, Temple University, Philadelphia, PA

Fel's Trainee Day, The Fels Institute for Cancer Biology and Molecular Genetics, Temple University, Philadelphia, PA

- **Journal publications.** Nothing to report
- **Books or other non-periodical, one-time publications.** Nothing to report
- **Other publications, conference papers, and presentations.**
- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

M. Raza Zaidi, PhD – no change

Jozef Madzo, PhD – no change

Jaroslav Jelinek, PhD – no change

Name:	Sarah Preston
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Ms. Preston has worked to complete the majority of the milestones noted in this submission. She has created the mouse crosses necessary as described in the application. She has also completed analysis of the NGS data-sets collected, as well as create the necessary research materials needed for this project such as genetically manipulated cell-lines and generation of methylation data-sets.*
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Nothing to report

- **What other organizations were involved as partners?**
  - Nothing to report
  - **Location of Organization:** *(if foreign location list country)*
  - **Partner's contribution to the projec**t *(identify one or more)*
    - **Financial support;**
    - **In-kind support** *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;
    - **Facilities** *(e.g., project staff use the partner's facilities for project activities)*;
    - **Collaboration** *(e.g., partner's staff work with project staff on the project)*;

- **Personnel exchanges** *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*; and
- **Other.**

8. **APPENDICES:** ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

********** ********** **ADDITIONAL NOTES:**

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